



Illuminating Cellular Systems Biology

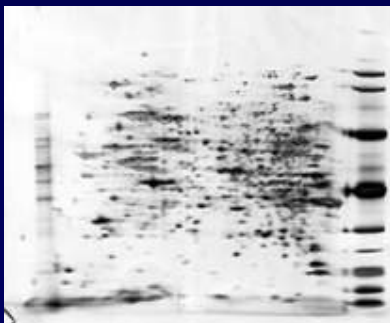
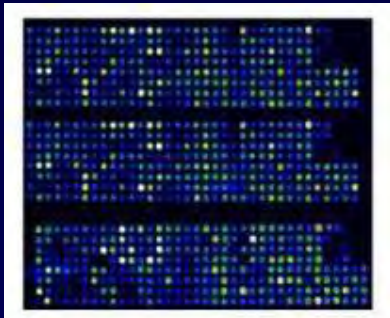
# Cellular Systems Biology: An Approach to Cytotoxicity Profiling

Computational Toxicology Forum  
May 23, 2007

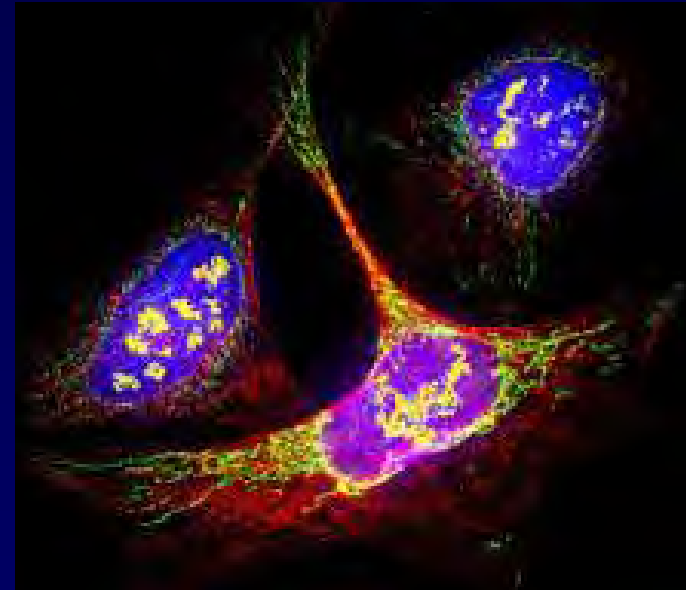


# Cellular Systems Biology

The cell is an integrated and interacting network of genes, proteins & metabolic processes that gives rise to function



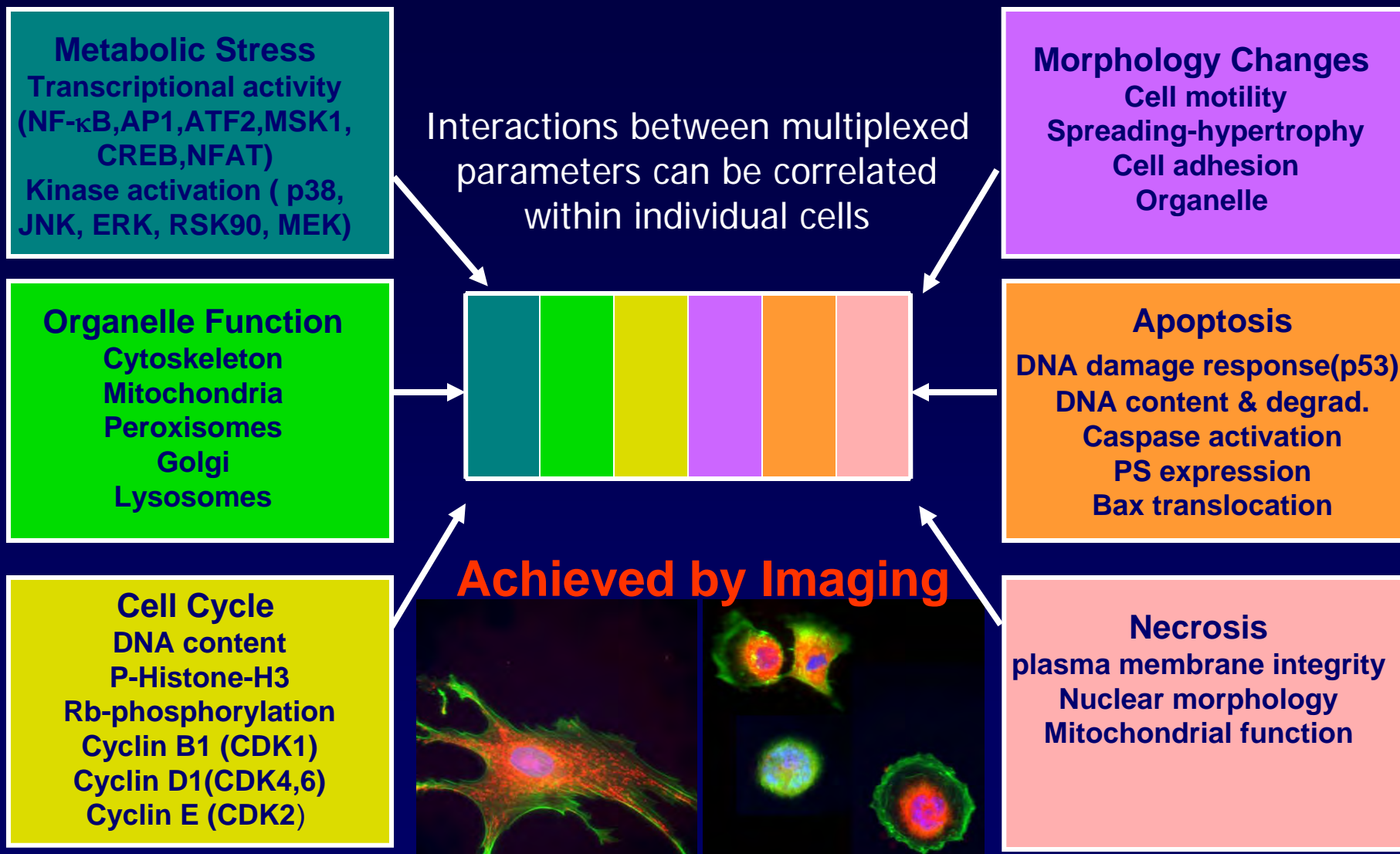
**Emergent Properties**



**Life!**  
& many cellular (dys)functions

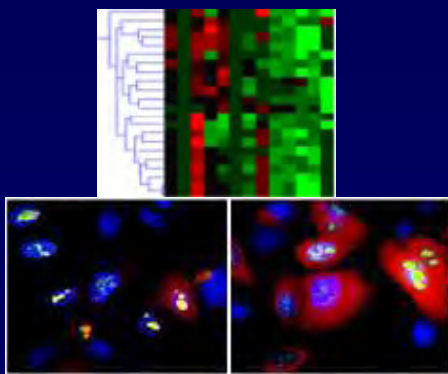


# Cytotoxicity Systems Profiling



# Why Cellular Systems Biology for Toxicity Testing?

- Intact, functional system
- Captures multiple MOAs



**Systems  
Relevance**

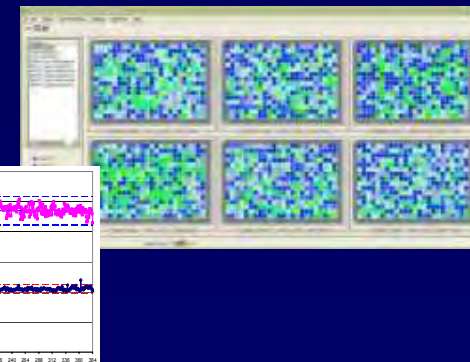


- Relevant species
- Metabolic capacity

**Integrated  
Content**

**Throughput**

- High Capacity
- Rigorous QC



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# Evolution of Toxicity Testing



Cellular Systems Biology



Toxicogenomics Profiling



HCS Assays\*



Uncorrelated Cell-Based Assays

Animal Models

\*O'Brien P *et al.*, *Arch Toxicol* (Apr 6, 2006)



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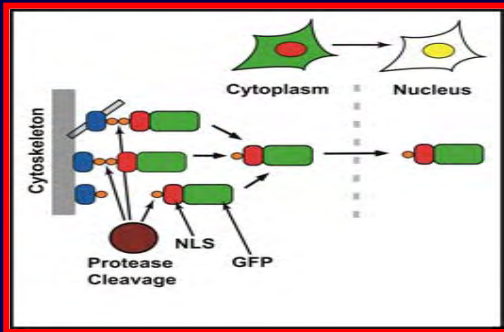
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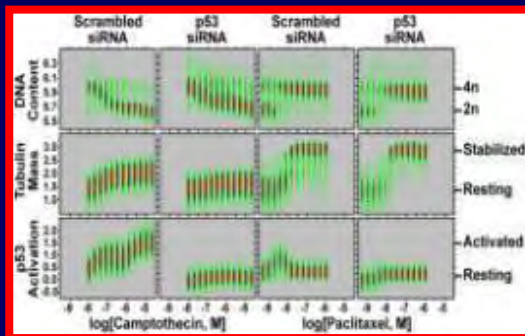
# Tools of Cellular Systems Biology



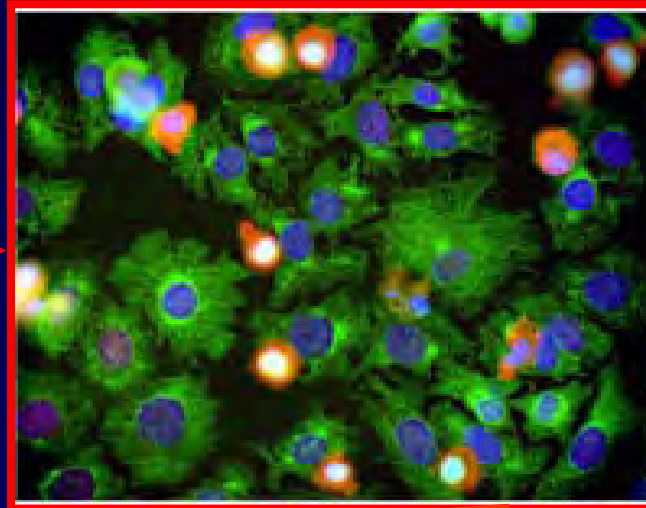
Imaging Detectors



Reagents



Informatics



Cell Models

**Systems Knowledge**



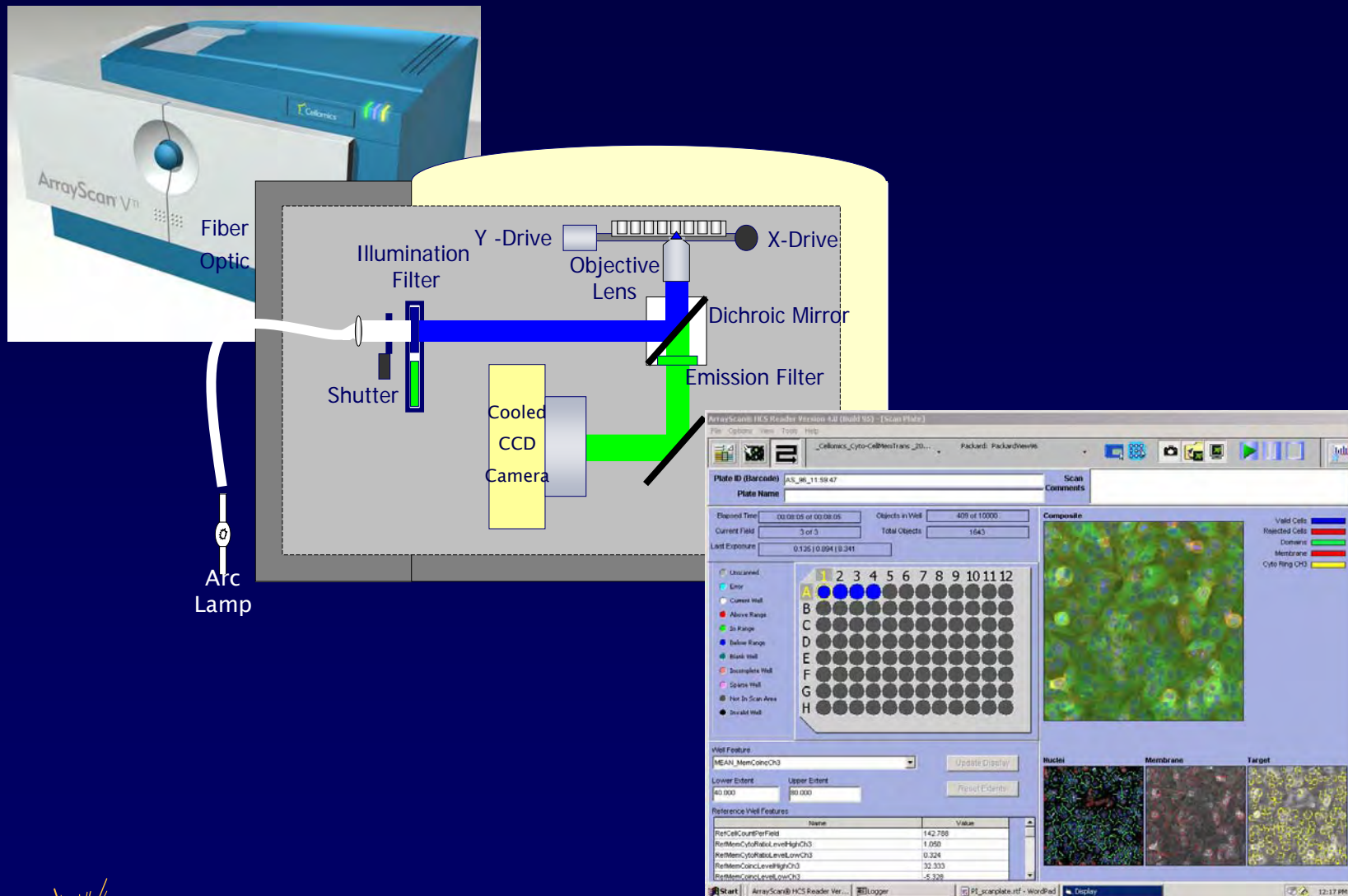
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# Imaging Platforms



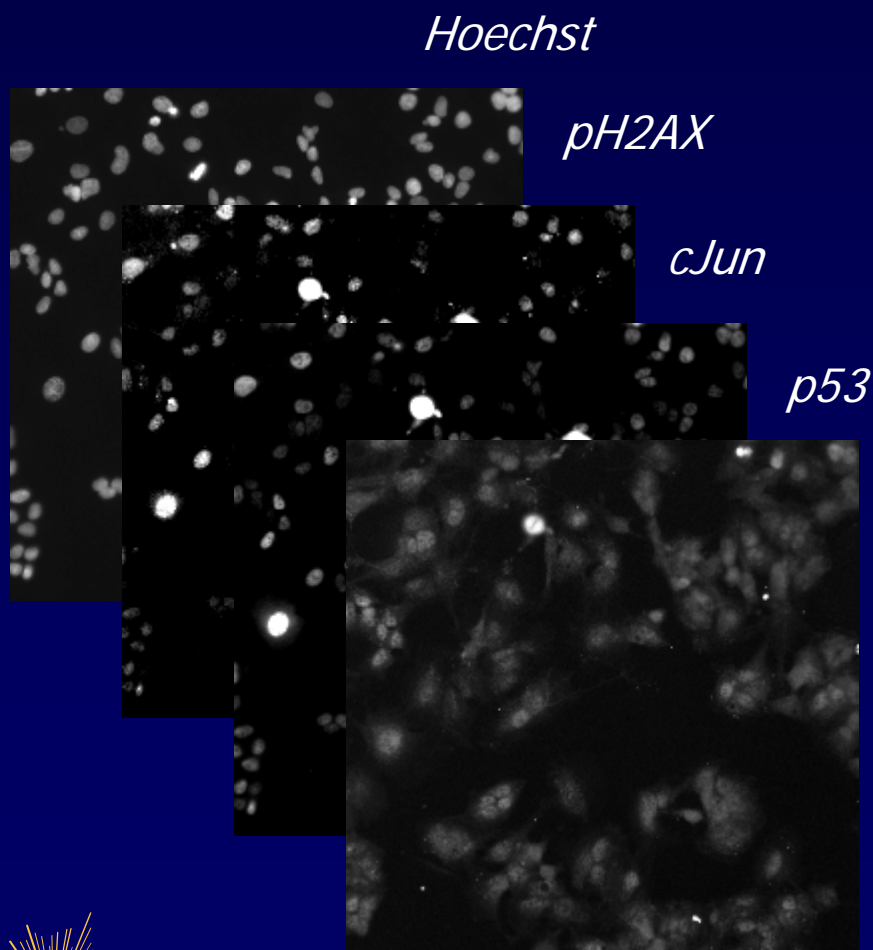
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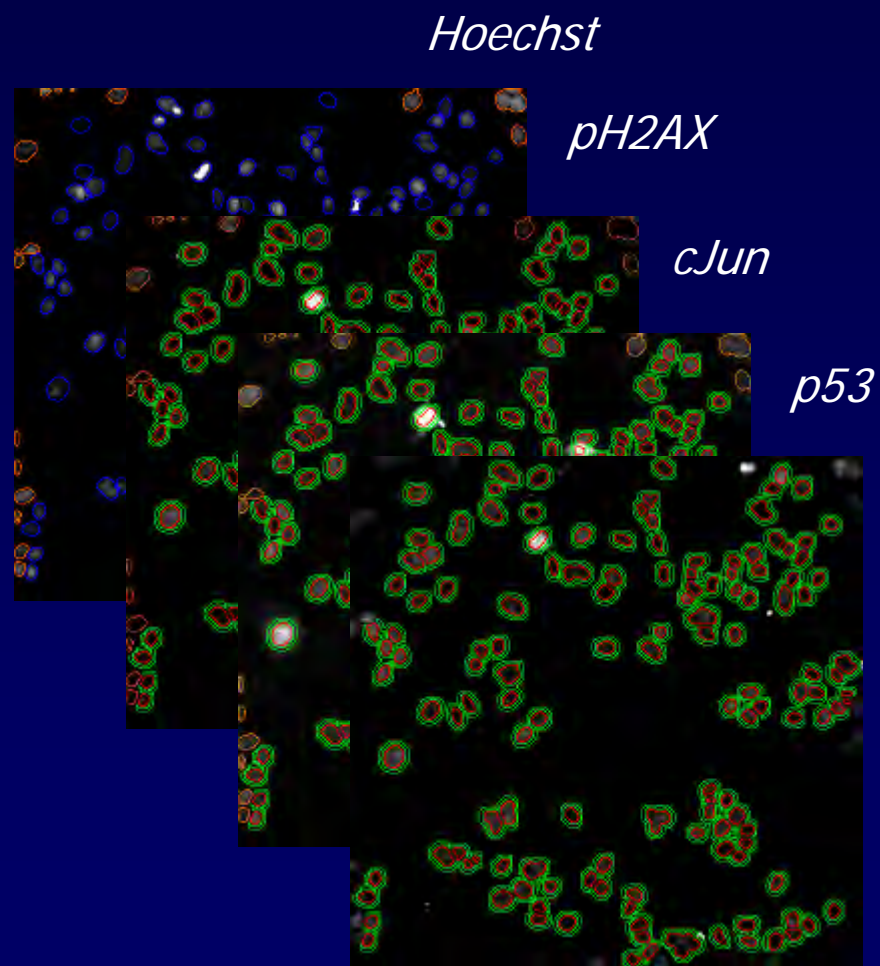
# Image Acquisition and Analysis

Measurements made in multiple compartments of *each* cell

## Raw Images



## Processed Images



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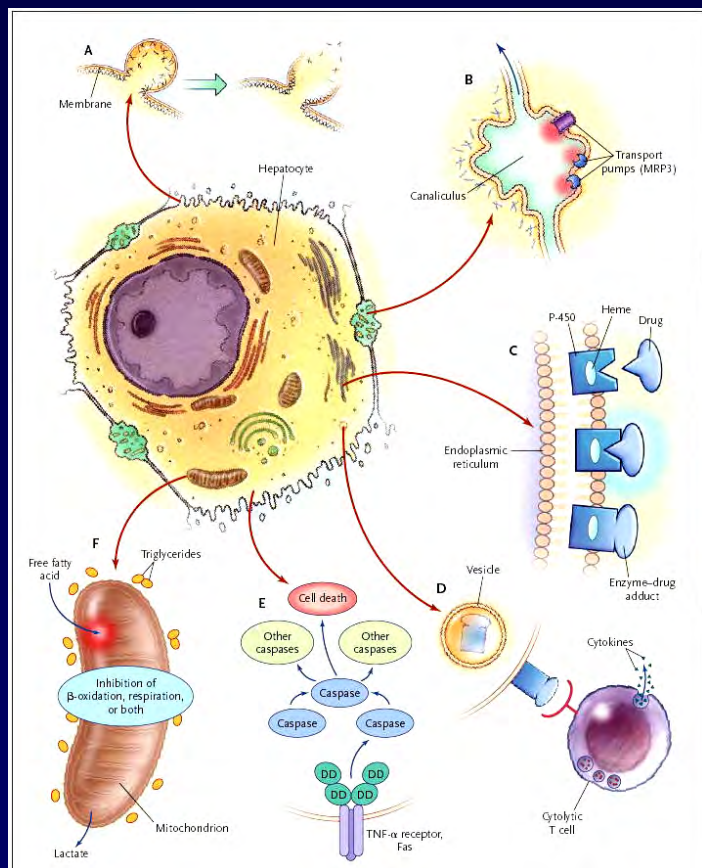


# Cellular Systems Biology: Cytotoxicity Profiling

- ✓ Hepatotoxicity (hepatocytes, HepG2 cells)
- ✓ Genotoxicity (micronucleus formation)
  - Neurotoxicity (neuronal cultures, PC12 cells)
  - Cardiotoxicity (cardiac myocytes)
  - Nephrotoxicity (cultures of renal tubules, nephrons)
  - Immunotoxicity (Jurkat cells, peripheral blood monocytes)



# Hepatotoxicity: Mechanisms & Markers



\* Adapted from Lee, WM. N Engl J Med 349 (5); 474 (2003)

- ✓ Disruption of intracellular calcium homeostasis, CSK disassembly and cell membrane perturbations
- ✓ Activation of apoptotic pathways
- ✓ Drug inhibition of mitochondrial function
- ✓ Peroxisome proliferation (rodents)
- ✓ Phospholipidosis, Steatosis
- ✓ Inhibition of transport pumps, bile acid accumulation (cholestasis)

P-450 system-generated high-energy reactions lead to drug-protein adducts

Enzyme-drug adducts serve as target immunogens for cytolytic attack



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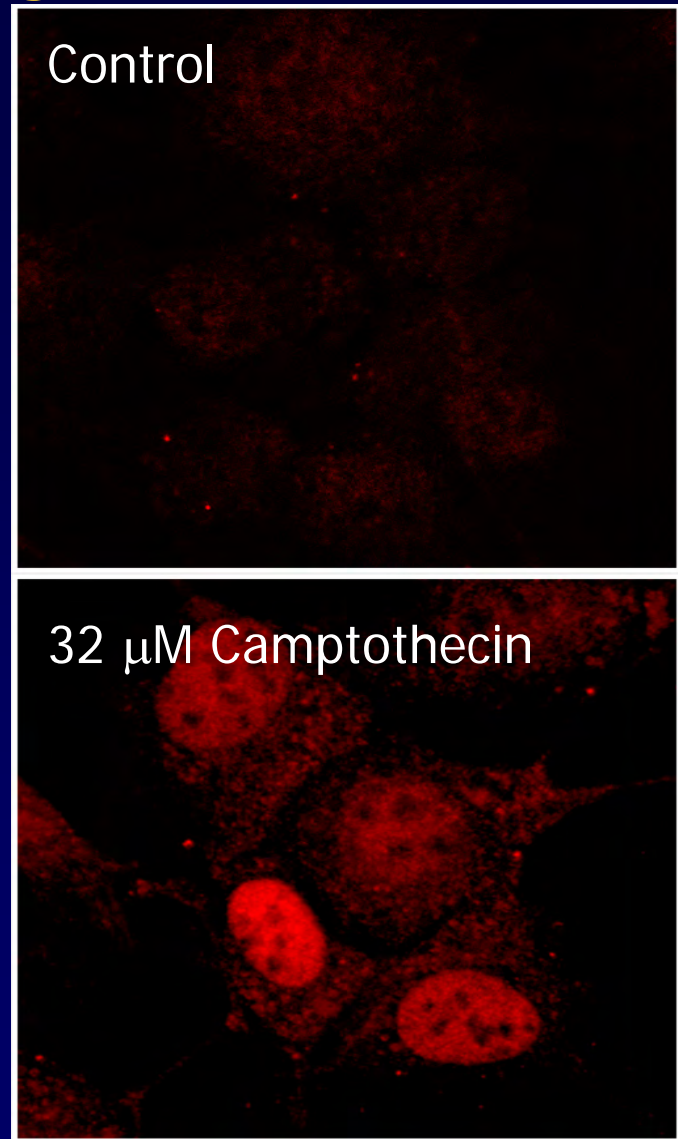
# Hepatotoxicity Parameters

- Apoptosis & necrosis
  - Cell cycle markers
  - DNA damage markers
- Metabolic stress
  - Oxidative stress (ROS)
  - Stress kinase activation
  - Mitochondrial function
- Organelle remodeling
  - CSK
  - Nucleus
- Peroxisome proliferation
- Phospholipidosis, Steatosis
- Cholestasis



# Apoptosis: DNA Damage Response

- Toxin-induced DNA damage activates several pathways, including the transcription factor p53 & the histone H2A.X
- p53 is activated via phosphorylation and nuclear translocation
- The DNA damage response can be measured in fixed cells by determining the Nuc:Cyto of p53 at 24hrs

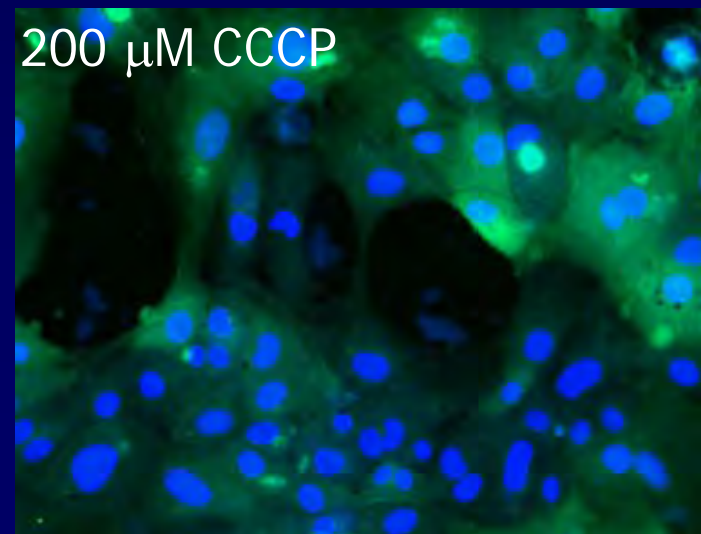
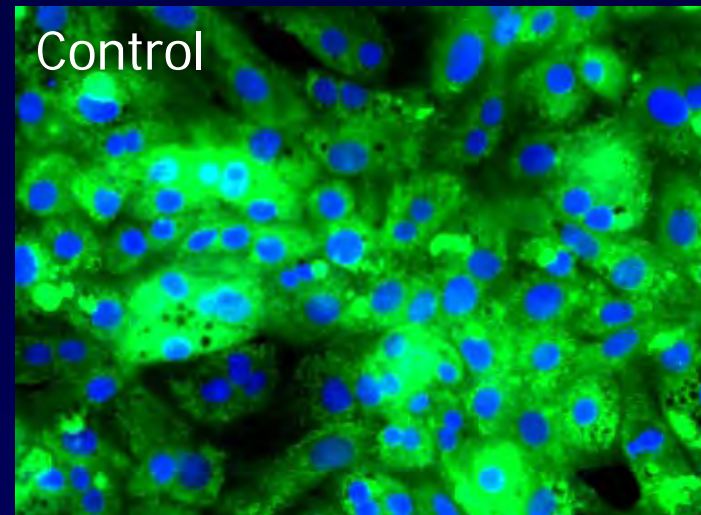


Rat hepatocytes – 24 hr



# Mitochondrial Function

- Chemicals can modulate mitochondrial function acutely (disrupt membrane potential & respiration). Chronic exposure can affect mitochondrial content
- Mitochondrial function can be visualized in both live and fixed cells via accumulation of fluorescent dyes that are sensitive to  $\Delta\Psi$ , e.g., Mitotracker Red, JC-1



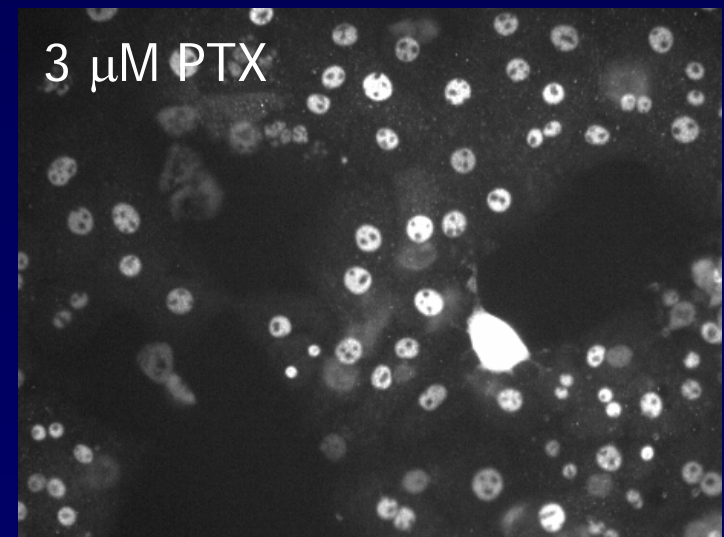
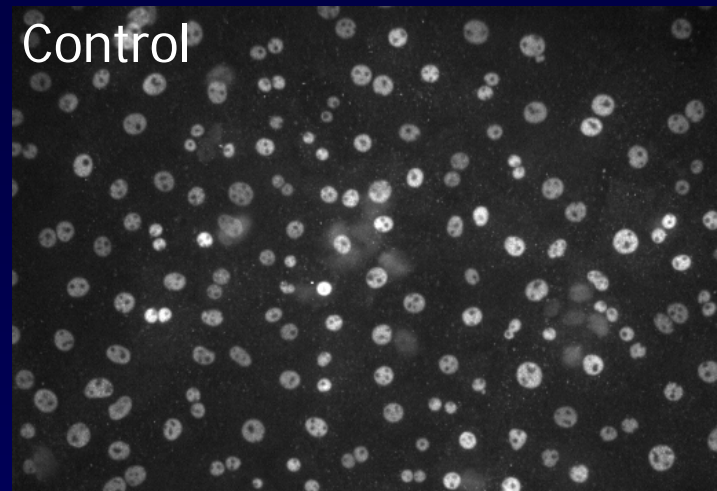
Rat hepatocytes – 24hrs





# Stress Kinase Activation

- Activation of cellular signaling pathways is one of the earliest markers of toxicity
- Kinases are rapidly activated via phosphorylation and or translocation, inc. the “stress” kinases p38, JNK, ERK, RSK90 MEK, etc
- Stress kinase activation (JNK) is measured with phospho-c-Jun Ab (nuclear intensity)

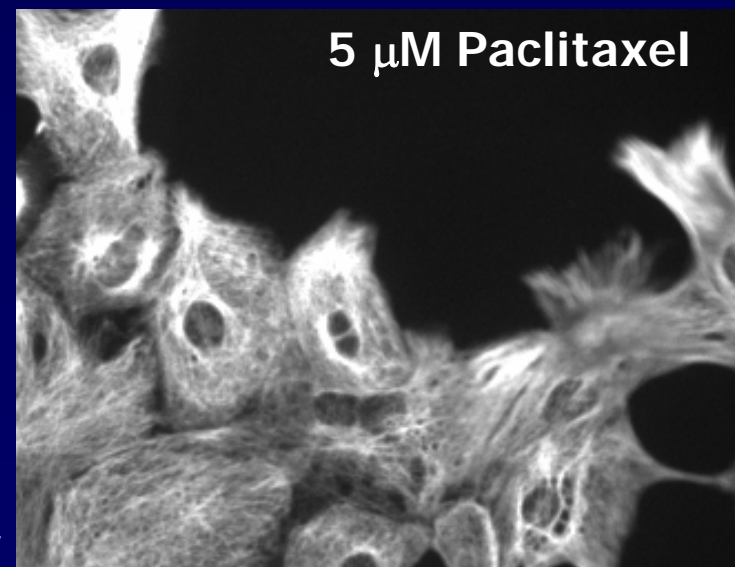
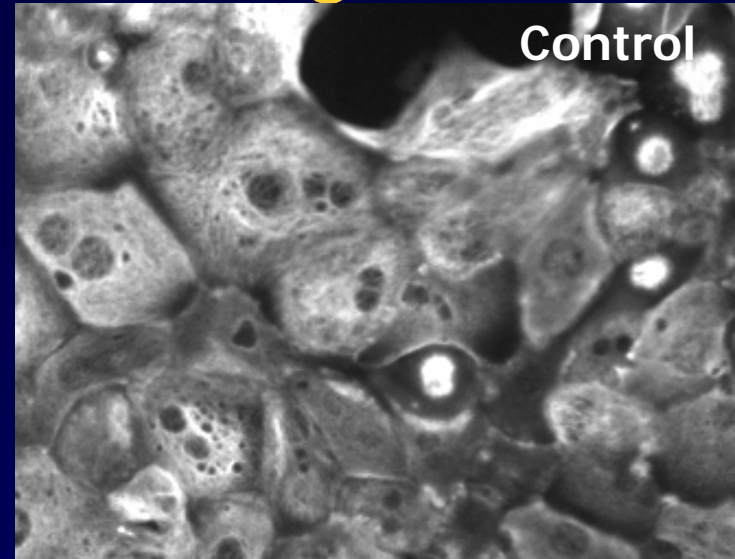


Rat hepatocytes – 24 hr



# Cytoskeletal Remodeling

- Toxins induce remodeling of the tubulin, actin & IF cytoskeleton
- Affects include altered cell shape, migration, & attachment either to the matrix or other cells
- Subtle remodeling affects inc., intracellular transport, signaling and the cell cycle
- The CSK is visualized in fixed cells with tubulin Ab labeling of total, non-extractable MT mass



Rat hepatocytes -24 hr



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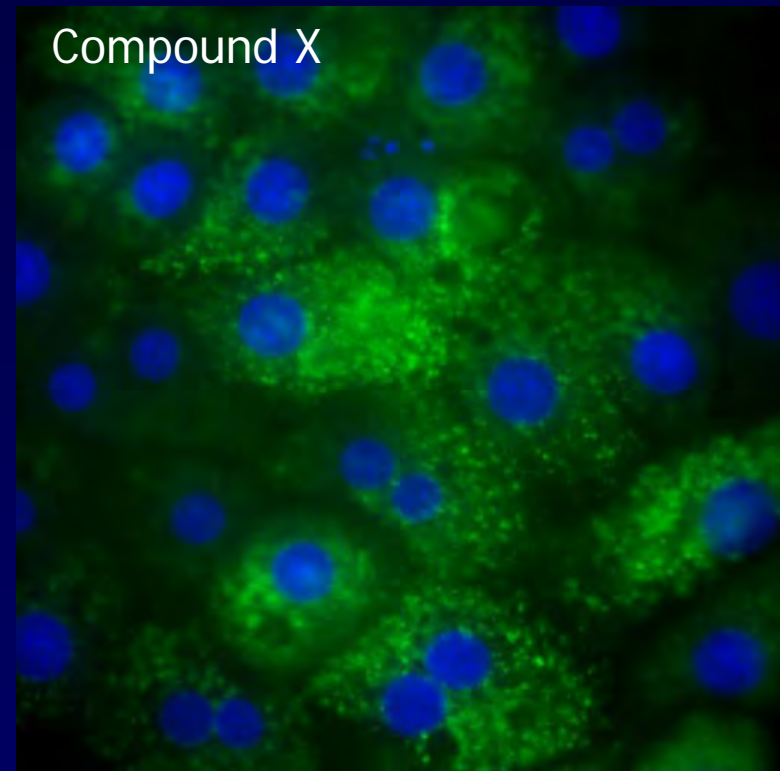
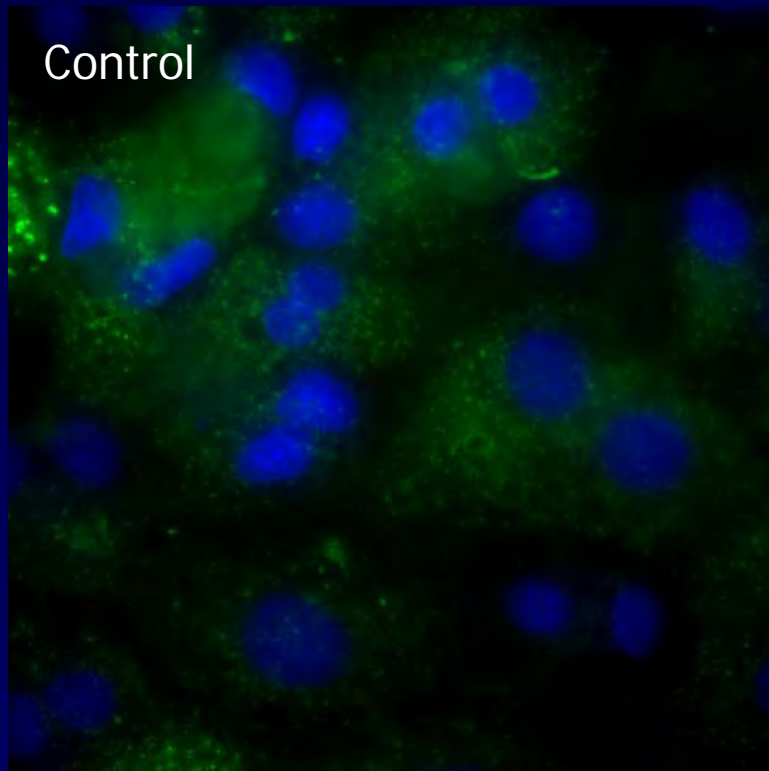
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# Peroxisome Proliferation

- Increase in peroxisome number
- Mechanisms for drug-induced PP
  - PPAR $\alpha$  agonists
  - Trans-activation of peroxisomal enzymes, hepatocellular proliferation, rodent liver hyperplasia
- Concerns with peroxisomal proliferation
  - diminishing concerns that PPs have human hepatocarcinogenetic potential, but FDA requires they be identified
  - humans display the hypolipidemic effects of PPs
  - EPA lists them as potential carcinogens
- HCS assays include measures of perox. mass



# Peroxisome Proliferation Assay



Rat hepatocytes, 48 Hrs of culture, PMP70 Ab



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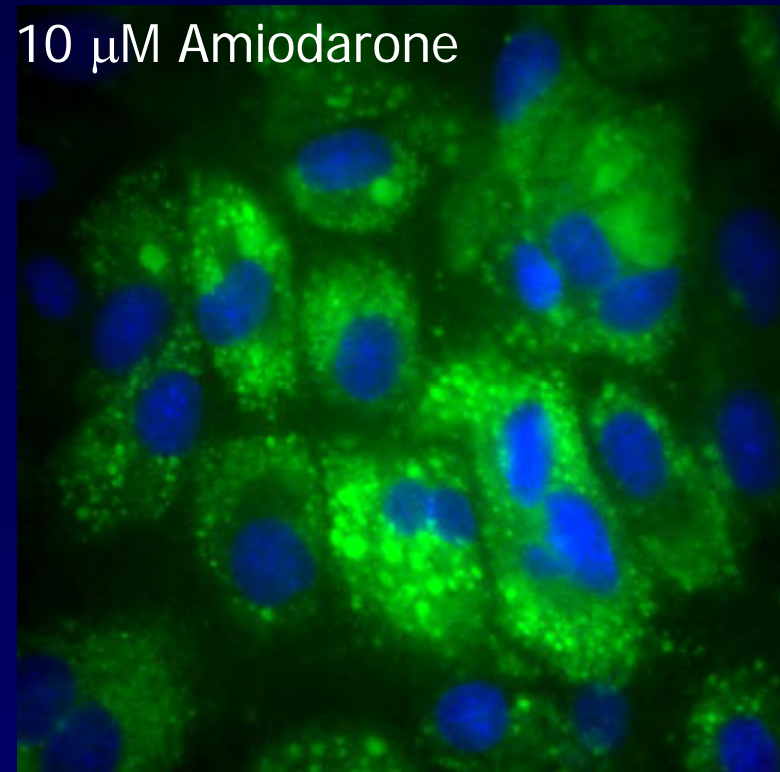
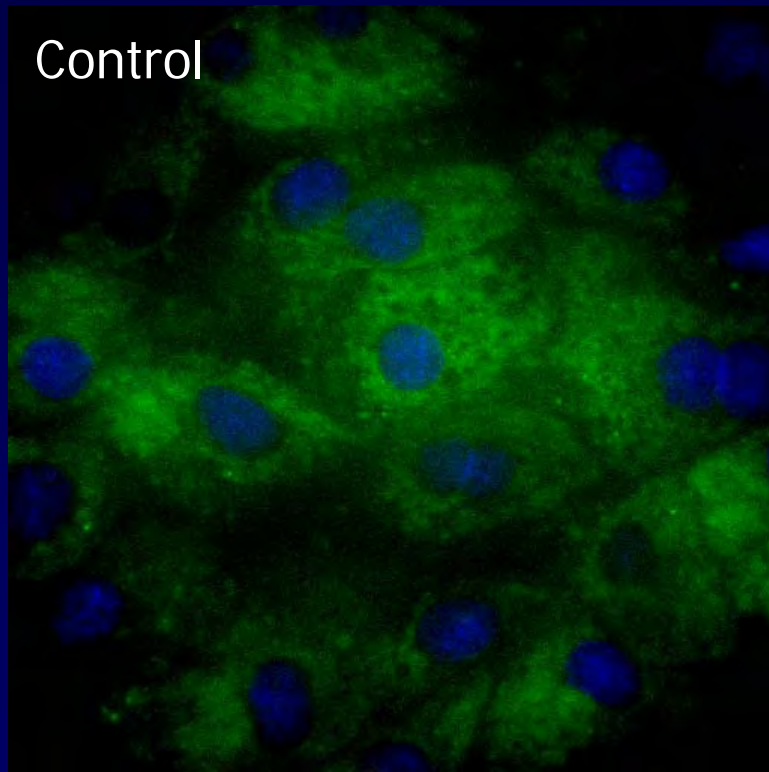
# Phospholipidosis

- Intracellular accumulation of phospholipids
- Mechanisms for drug-induced PL:
  - Inhibition of lysosomal phospholipase activity
  - Increase in phospholipid synthesis
- Concerns with PL induction:
  - Disruption of cell phospholipid pools
  - Intracellular drug accumulation
- Assays include: measures of lysosomal mass and fluorescent phospholipid accumulation





# Phospholipidosis Assay



Rat hepatocytes, 48 Hrs of culture, LysoTracker Green



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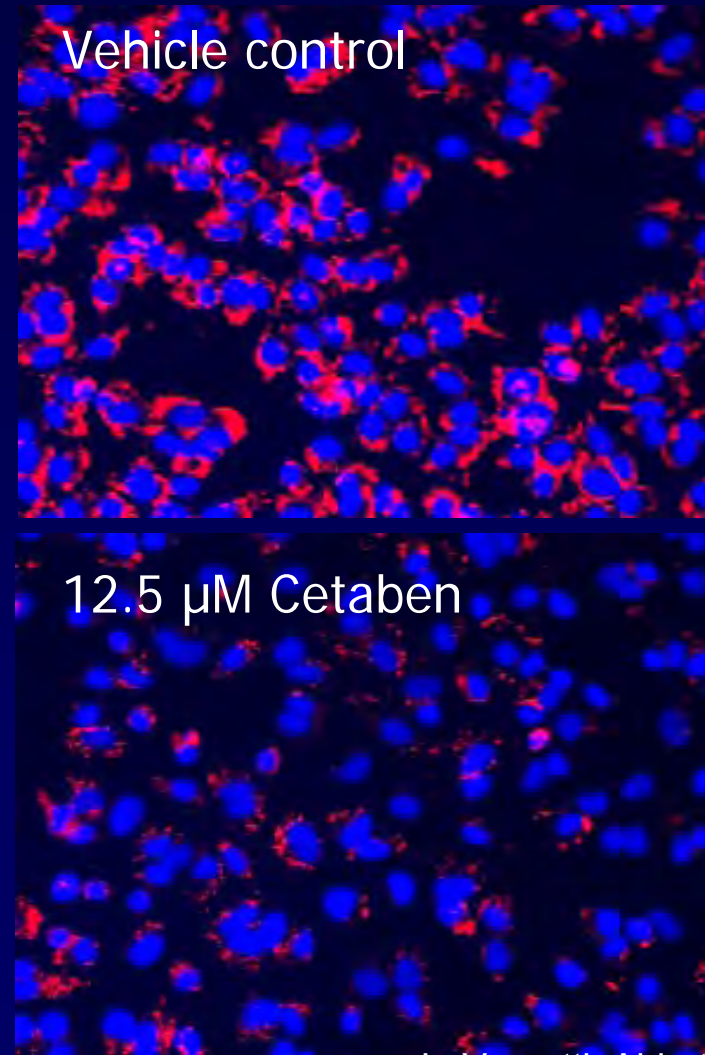
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# Steatosis Assay

- Chemical agents can disrupt cellular lipid metabolism resulting in triglyceride accumulation
- 2 hr serum & insulin starvation followed by Oleic Acid treatment (18hr, triglyceride formation)
- Neutral fats visualized with Oil-Red-O

Hep G2 cells

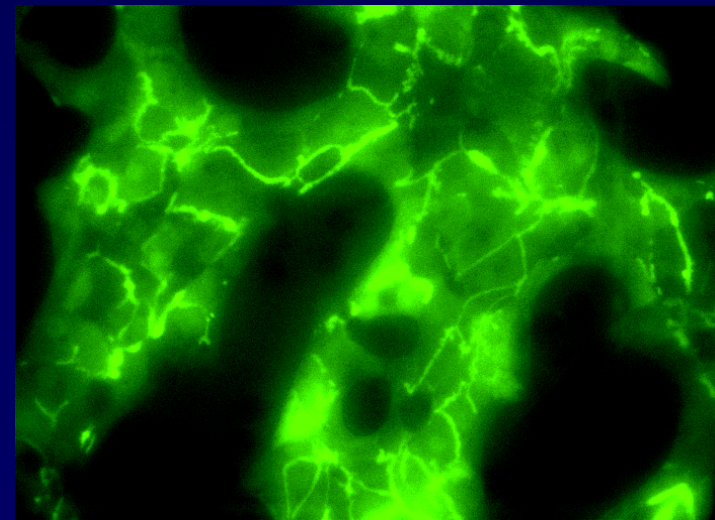
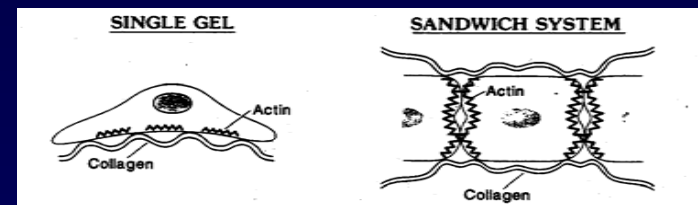


L. Verneti, Abbott



# Cholestasis

- Chemicals can induce cholestasis – a reduction in bile production and/or flow from the liver
- Sandwich hepatocyte cultures generate canalicular spaces lined by transmembrane pumps
- Cholestasis can be measured in vitro by detection of pump content or activity



Rat hepatocytes



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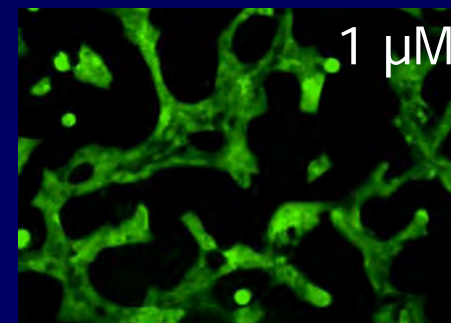
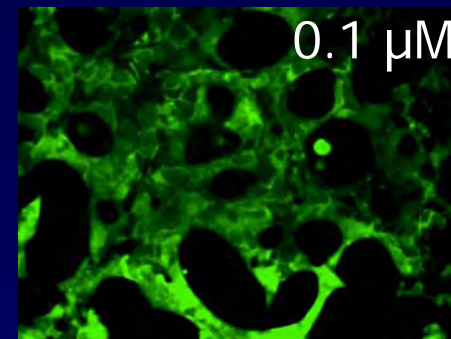
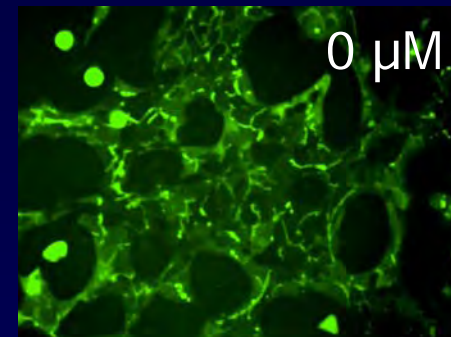
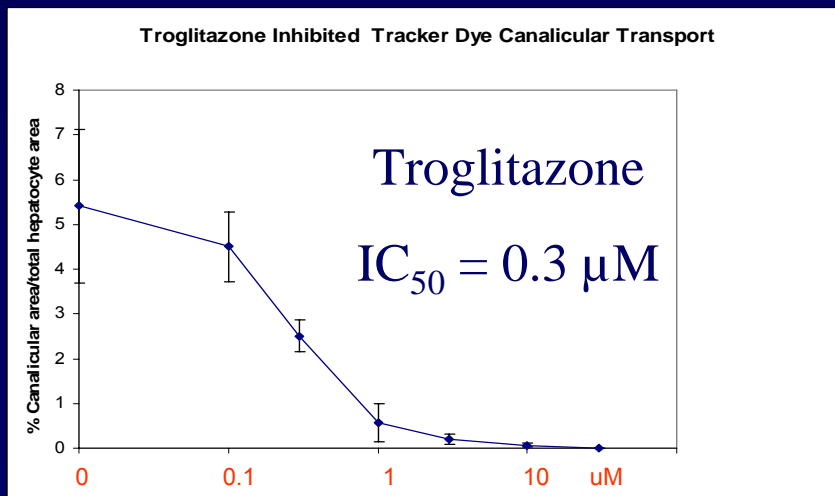
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# Cholestasis Assay

- 5 day sandwich cultures (Matrigel overlay) rat hepatocytes
- Cell Tracker Green Dye (CDF)
- Incubate 15 min, live read
- Measure % Cannalicular area



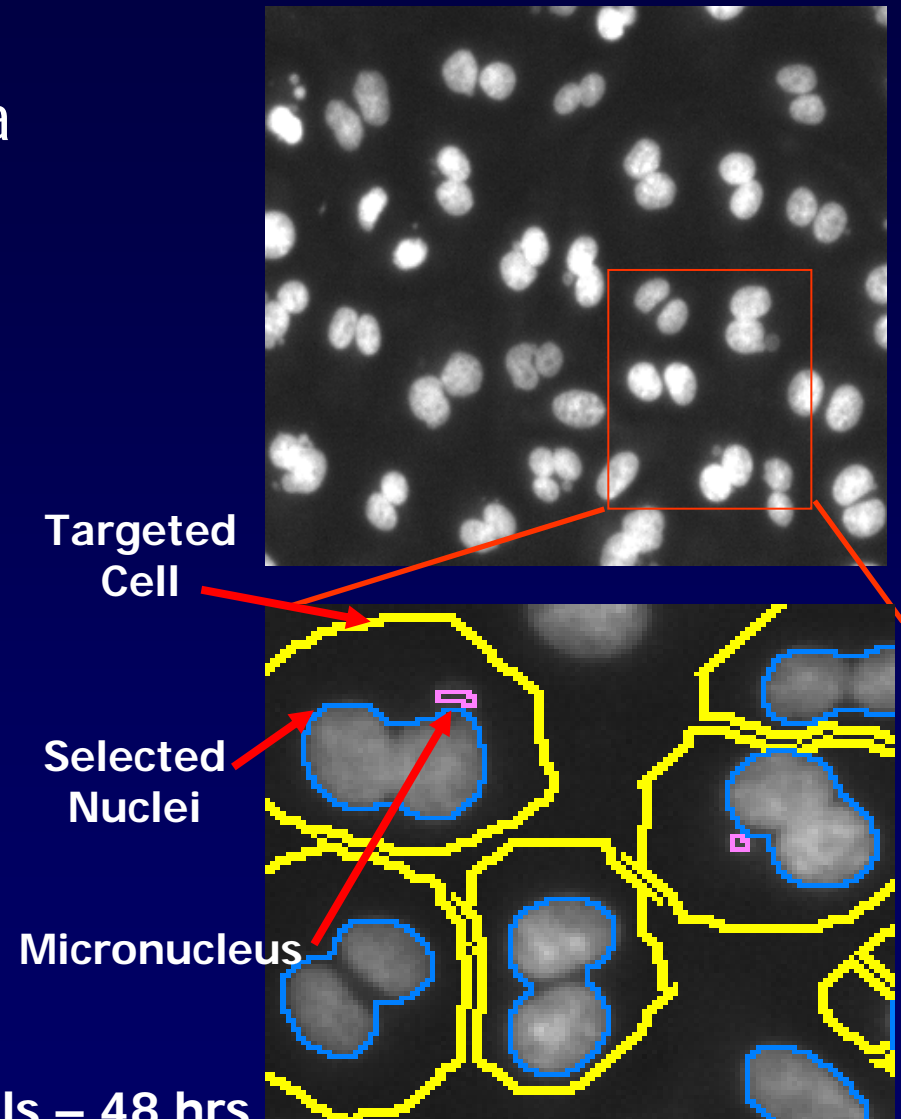
L. Vernetti, Abbott





# Automated Micronucleus Detection

- Micronucleus induction is a hallmark of genotoxicity
- Micronuclei arise from abnormal chromosome structure &/or segregation
- Cells are treated with test agent (20hrs) & then cytochalasin D (28hrs) to induce a cytokinesis block
- Cells are fixed & permeabilized. Micronuclei & nuclei are detected with a DNA-selective dye (Hoechst)



CHO cells – 48 hrs

Cellomics



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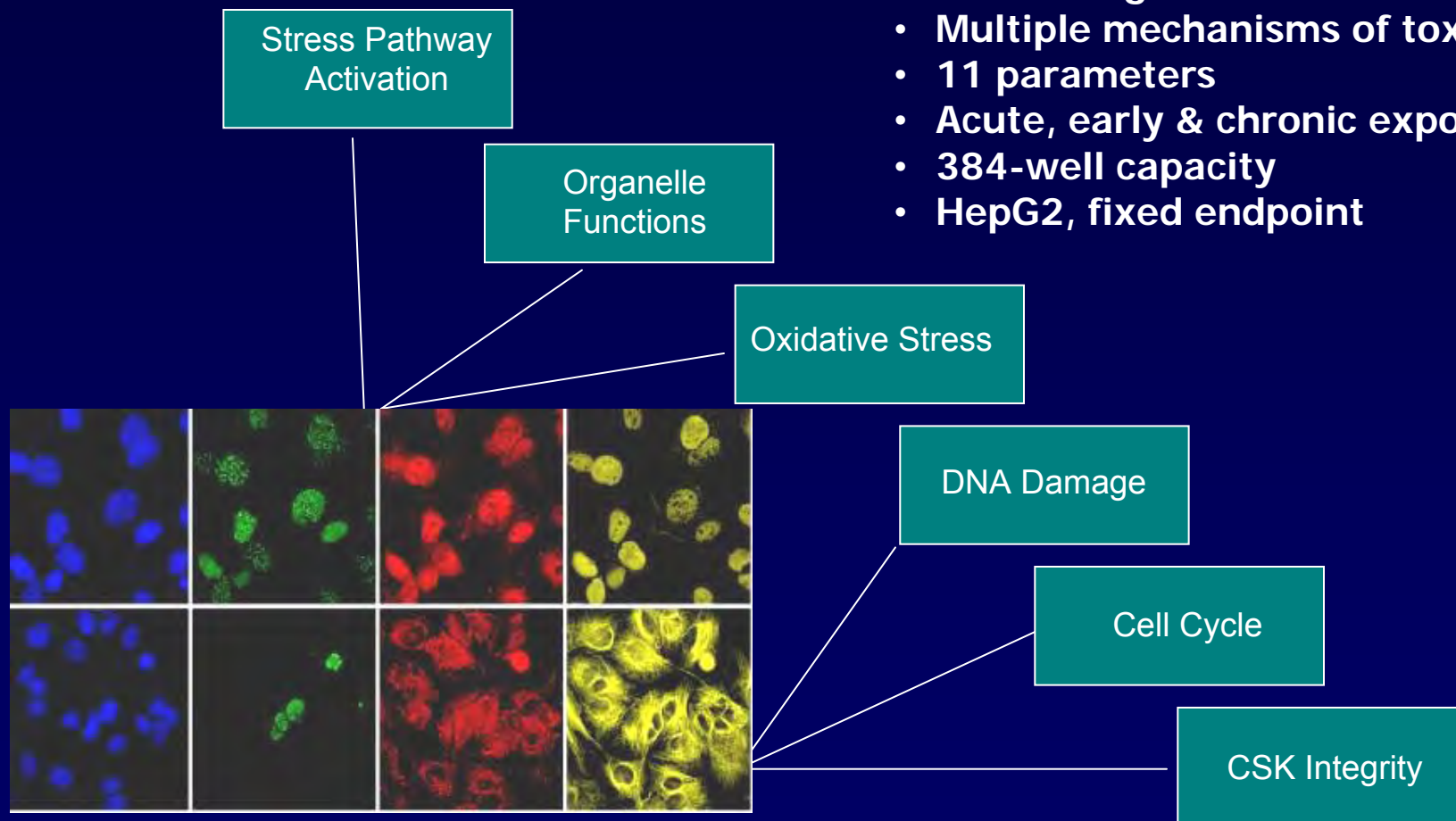
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# Multi-parameter Cytotoxicity Assays

## Panel 1 design\*:

- Multiple mechanisms of toxicity
- 11 parameters
- Acute, early & chronic exposure
- 384-well capacity
- HepG2, fixed endpoint



\*Cellumen patents pending



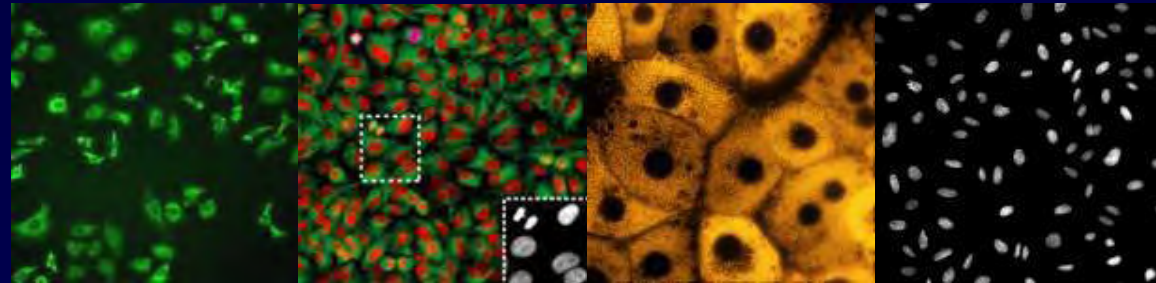
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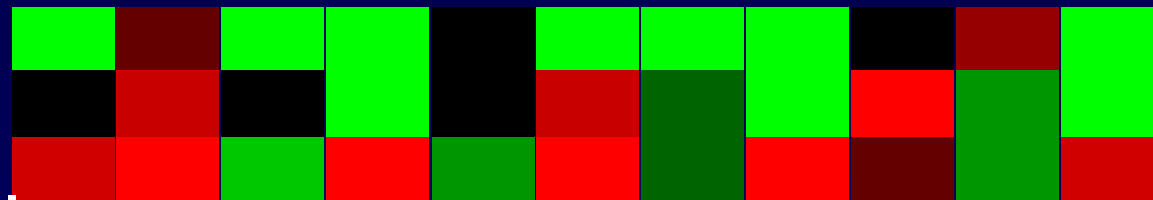
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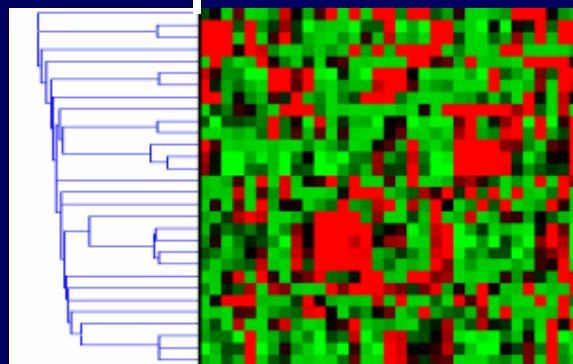
# Cytotoxicity Profiling: HepG2 Panel



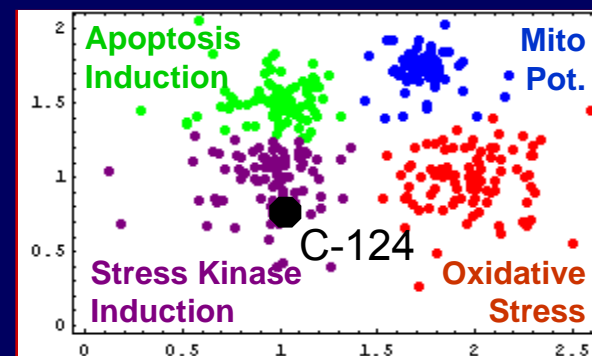
Acute Profile  
Early Profile  
Chronic Profile



Clusters of compounds  
based on  
CellCiphr  
Profiles



Library of CellCiphr Profiles



Compound Classification



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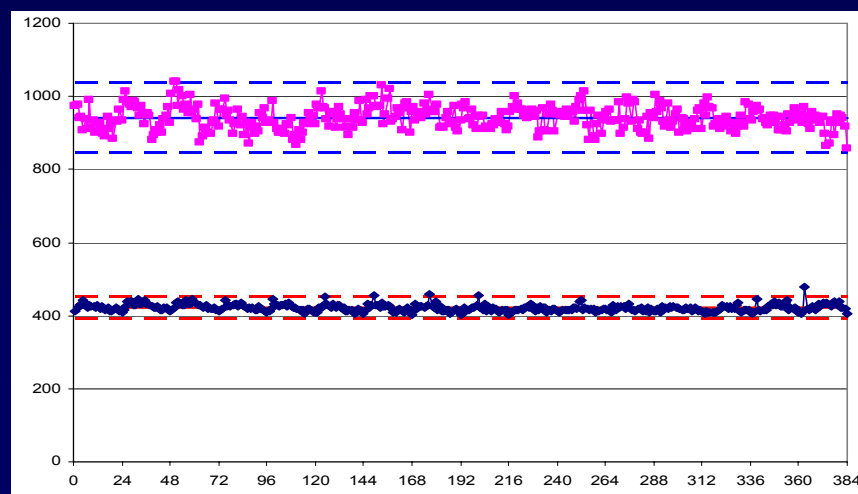
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# High Capacity Format

	Mean	CV	S/B	Z'	Drift
Day 1 Min	421	2.3	2.23	.76	2%
Day 1 Max	941	3.4			2%
Day 2 Min	421	3.2	1.75	.63	1%
Day 2 Max	736	3.4			4%

384-well format  
maximizes capacity

Max  
Min  
Day 1



Oxidative Stress Response



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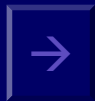
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# Quality Control

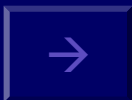
Parameter	Positive Control	Z'
Stress Pathway	Anisomycin	.78
Oxidative Stress	Camptothecin	.7
Mitochondrial Function	CCCP	.55
Mitochondrial Mass	CCCP	.35
Cell Loss	Camptothecin	.56
Cell Cycle	Paclitaxel	.54
DNA Degradation	Paclitaxel	.6
Nuclear Size	Paclitaxel	.63
DNA Damage	Camptothecin	.43
Mitotic Arrest	Paclitaxel	.63
Cytoskeletal Integrity	Paclitaxel	.5

## Cytotoxicity Panel One – Z' Summary



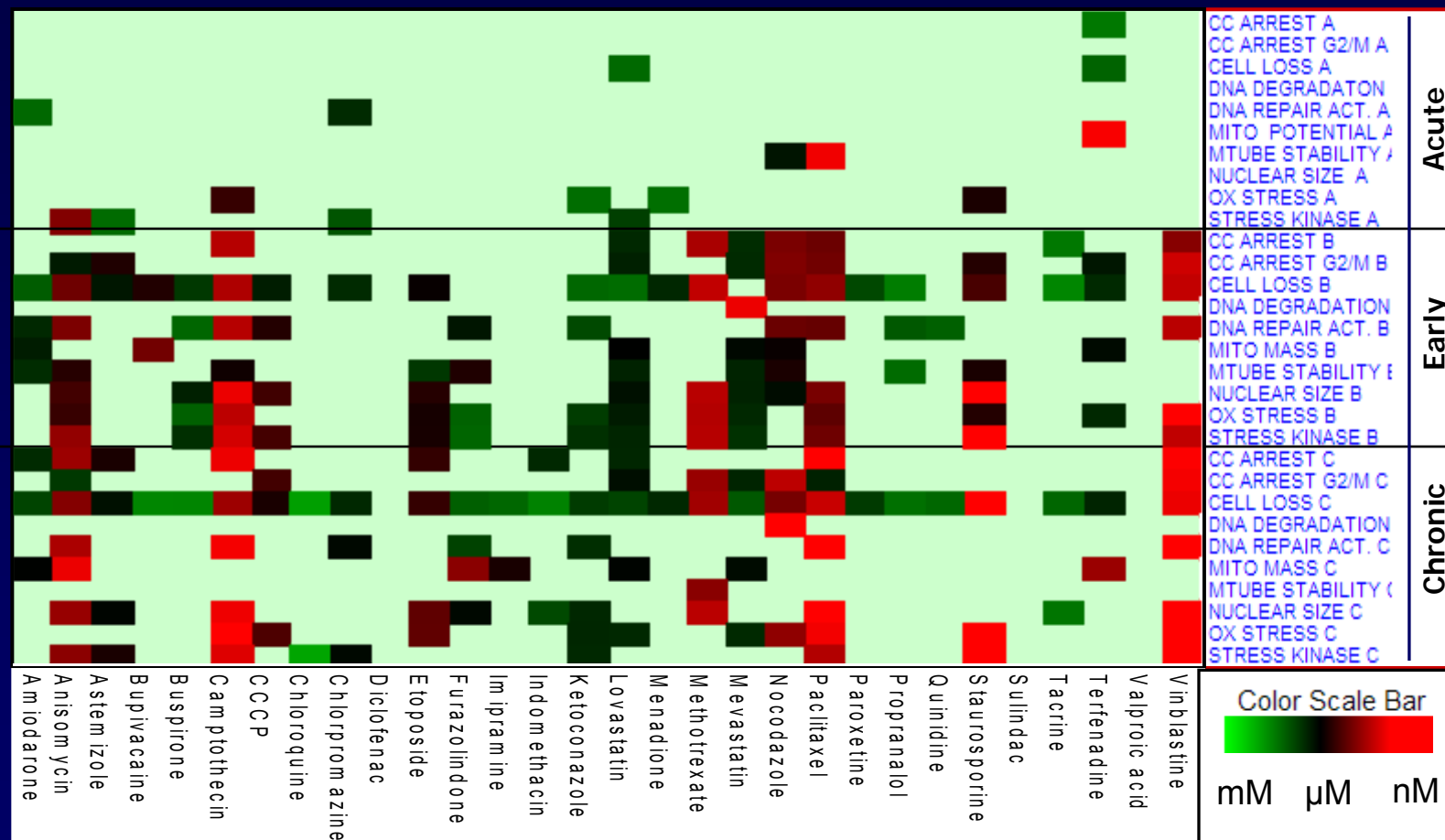
# Initial Validation Cassette

Broad Spectrum		Compound	# Assays	Compound	# Assays	Narrow Spectrum	
Broad Spectrum	{	Lovastatin	19	Buspirone	6	{	Narrow Spectrum
		Paclitaxel	18	CCCP	6		
		Anisomycin	18	Propranalol	5		
		Mevastatin	16	Tacrine	5		
		Camptothecin	16	Paroxetine	4		
		Vinblastine	14	Bupivacaine	3		
		Nocodazole	13	Chloroquine	3		
		Staurosporine	12	Chlorpromazine	3		
		Terfenadine	11	Indomethacin	2		
		Etoposide	11	Menadione	2		
		Amiodarone	10	Quinidine	1		
		Astemizole	9	Sulindac	-		
		Ketoconazole	9	Imipramine	-		
		Furazolidone	8	Diclofenac	-		
		Methotrexate	8	Valproic acid	-		

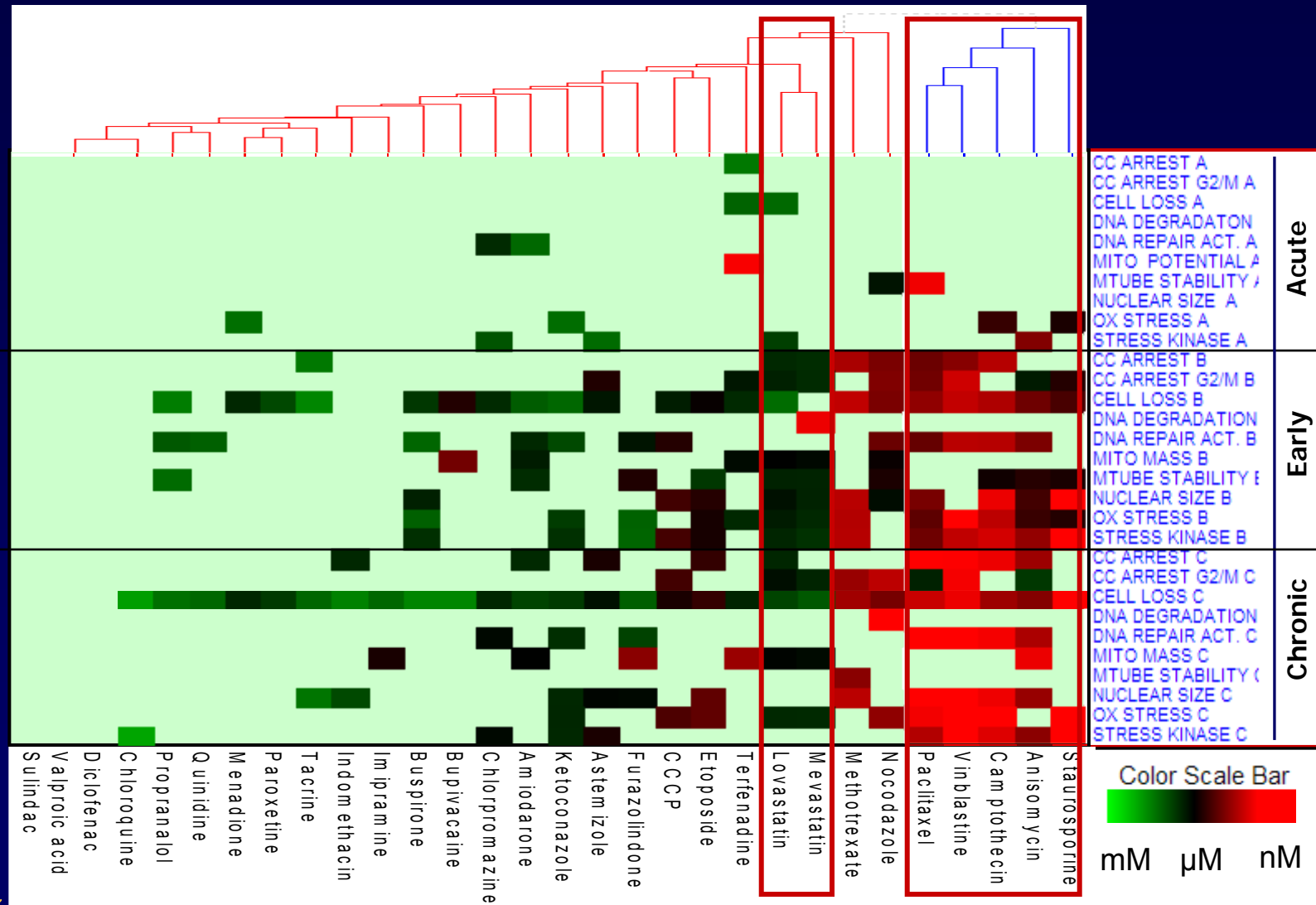




# CellCiphr™ Validation Cassette Profile



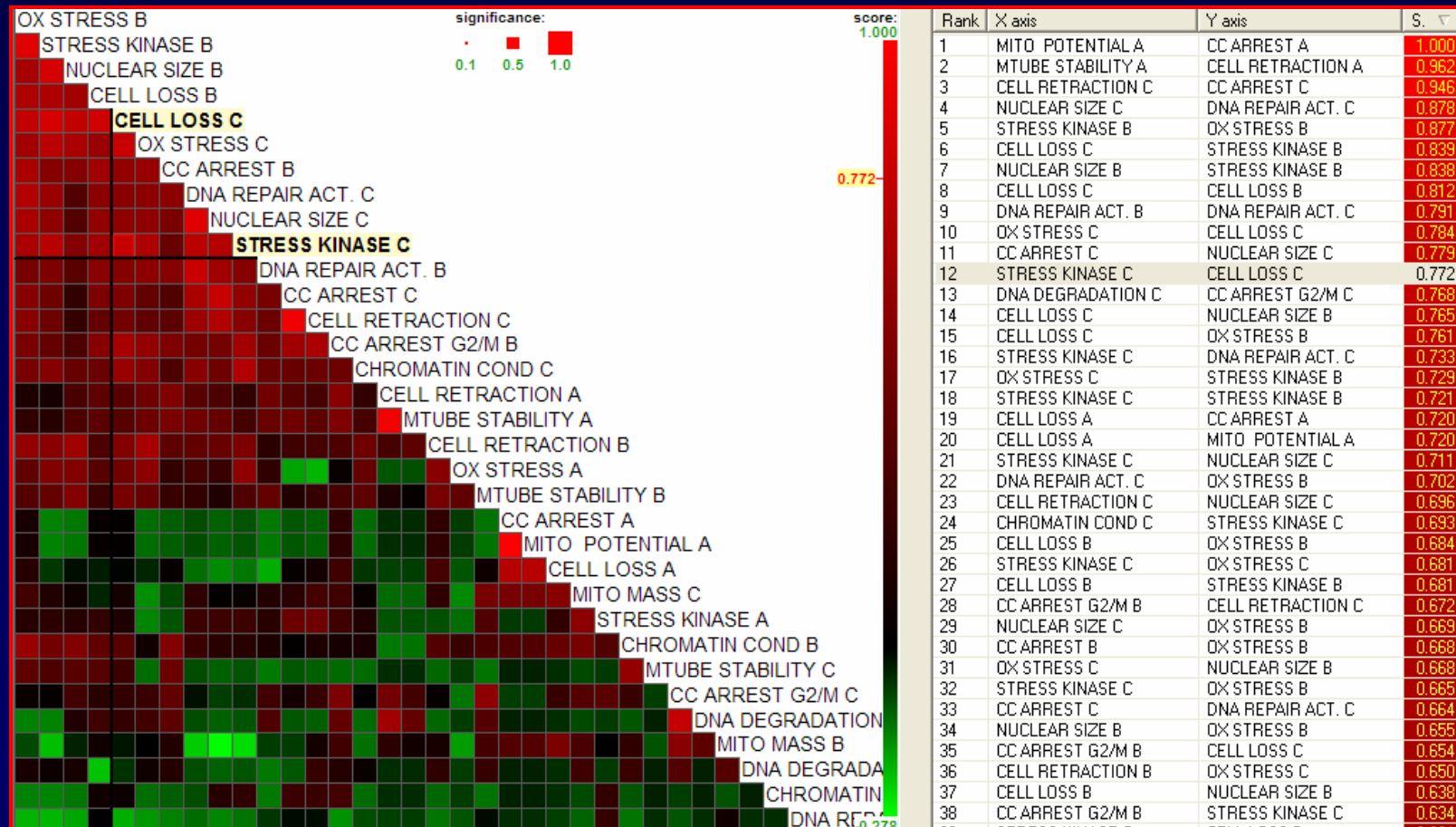
# Validation Cassette Profile - Clustered



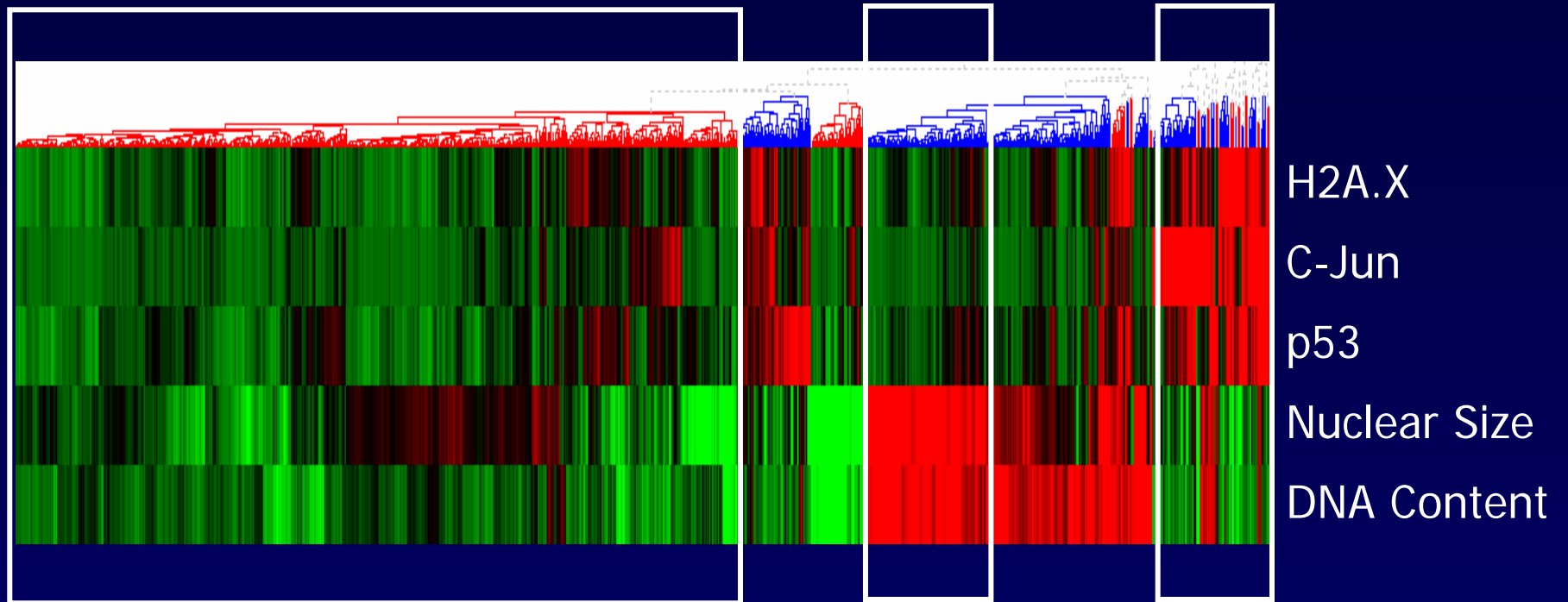
# Correlations Between Cell Population Features

Correlation Map

Most Significant Correlations



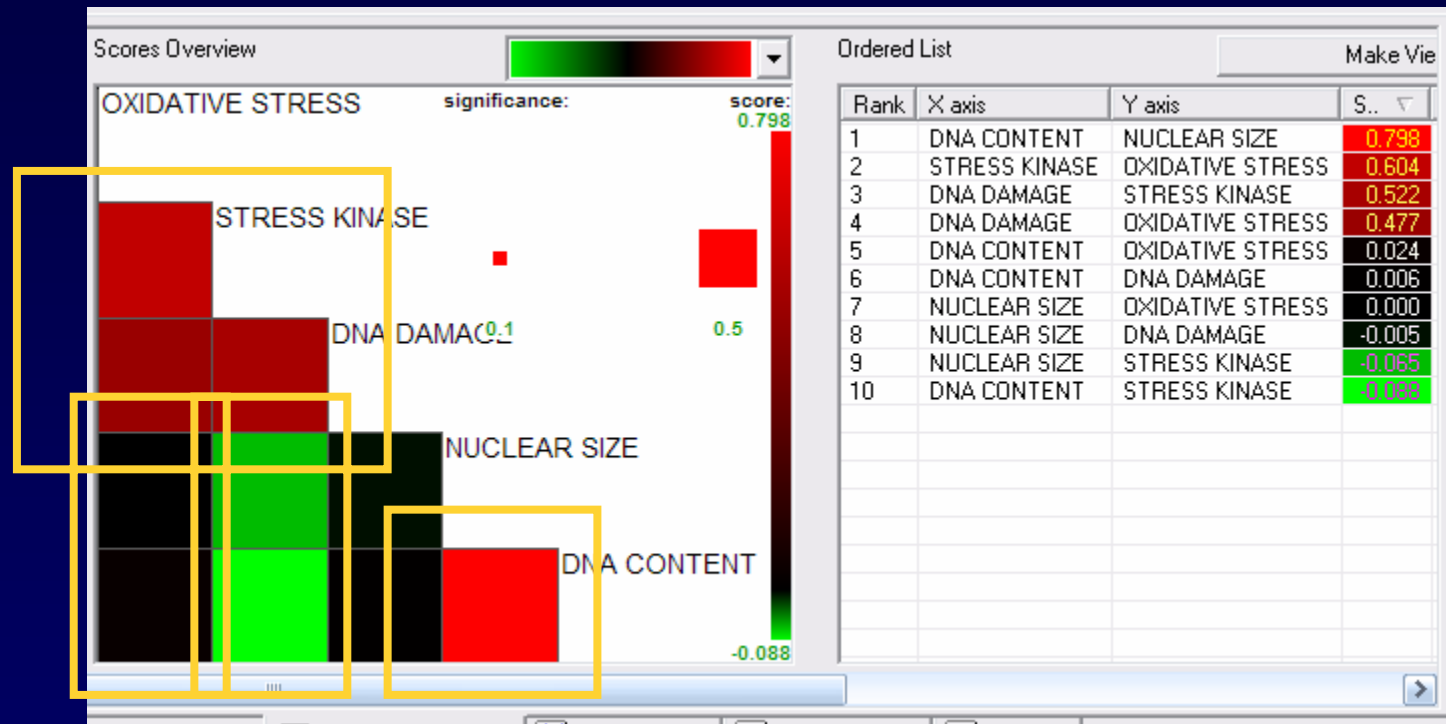
# Profile: Single Cell Heat Map



- HepG2 cells treated with 100nM camptothecin (sub-toxic inflection pt) for 72 hours
- Each line represents a single cell in 1 well
- Several sub-populations of cells apparent



# Correlations Between Activation of Pathways in Individual Cells



- HepG2 cells treated with 100nM camptothecin for 72 hours
- Several patterns of correlation *between* parameters are apparent





# Conclusions

- The Cellular Systems Biology approach to profiling environmental chemicals offers several advantages to both traditional toxicity testing and HTS
  - *Relevant* systems - species, cell type and metabolic capacity
  - Independent of mechanism - multiple targets & target classes measured simultaneously
  - Throughput – 384 well capacity enables measure of multiple doses & time points
  - Captures single cell & population responses



# Acknowledgements

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Thank you!

